Ring around the heart: an unusual feature of the crocodilian central circulatory system.

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ABSTRACT

We have characterized the right (RAo) and left aortae (LAo) and their interconnections through the Foramen of Panizza (FP) and anastomosis (AN) as forming a circulatory 'ring around the heart'. We look at a couple of situations where postulated blood flow through the FP, both R→L and L→R could equally well be served by flow around the ring. Closure of the LAo not only prevents the pulmonary by-pass shunt but also the possibility of oxygenated blood reaching the gut through the FP. Section of the RAo, below the origin of the carotid artery, will severely restrict flow of oxygenated blood to the hind part of the body. LAo section in alligators had no effect on growth or energy conversion compared with sham operated animals indicating little selective advantage for retention of bulk flow of venous blood to the body. RAo section in caimans revealed marked effects on growth and energy conversion but too few animals were available to claim statistical significance. Nevertheless, caimans with the RAo sectioned grew much slower compared with animals with the LAo cut. The effect was generalized with no differences in the proportionate relations between head and body measurements. We speculate that the ring around the heart will function just as well as flow through the FP in effecting R→L or L→R blood transfer in the central circulation of crocodilians.

Key words: alligators, caimans, shunting, selective advantage, growth, conversion rates, foramen of Panizza, anastomosis.

Introduction

> *"each epoch has had its peculiar crocodiles"*
>
> T.H.Huxley (1868)

Among vertebrates, crocodilians not only have the most complex anatomy of the heart and outflow channels but possibly the most functionally sophisticated (Grigg 1991, *improved*). Functional sophistication seems an unlikely epithet to describe a cardiovascular system in which the heart itself is completely divided, like that of a bird or mammal. The right ventricle (RV), however, not only gives rise to the pulmonary artery (PA) but also the left aorta (LAo) through which venous blood can by-pass the lungs and be shunted back to the body (Fig 1). The left ventricle (LV) gives rise to only a single vessel, the right aorta (RAo). In addition, the aortae carrying oxygenated and deoxygenated blood are connected twice; just outside the heart, within the enlarged truncal region, by the foramen of Panizza (FP; Panizza 1833) and behind the heart by an anastomising vessel (AN). The caliber of both the AN and FP is variable and can be changed pharmacologically (Karila *et al* 1995) as well as *in vivo* with dramatic effects on LAo flow patterns (Jones 1996). The AN is orientated with the connexion to the LAo being upstream of its junction with the RAo suggesting that the primary course of blood is from the LAo to the RAo (Fig 1). Cardiovascular references are frequently used as descriptors for roadways so, using the reverse analogy, the aortae and their connexions form a ring road around the heart with two stoplights sited in regions which also have variable numbers of lanes (FP and AN).

What might the purpose of ring blood flow be? There have been a couple of suggestions in the literature. Farmer *et al* (2008) claim that shunting in alligators always occurs in the post-prandial period and, since the gut is a highly aerobic organ, oxygen rich blood must flow from the RAo to LAo to provide oxygen to the gut for digestion. Grigg (1991) hypothesized about the other side of the coin with venous blood flowing through the FP in the 'reverse' direction, from LAo to RAo. Grigg (1991) envisaged that in prolonged anaerobic dives, when the circulation is largely restricted to the brain and heart, oxygen stored in the lungs could be metered out to support these organs by opening the pulmonary circulation to flow periodically. In the periods when the pulmonary circulation is closed there will be no venous return to the left side of the heart, so the only blood moving from the heart would be carried by the LAo. Transferring venous blood to the RAo through the FP and hence to the brain and systemic circuit will perhaps be a 'life-saver'! Both Farmer *et al* (2008) and Grigg (1991) envisaged flow crossing from one side to the other through the FP but flow around the ring will serve equally well as a 'life-ring' and for digestion.

Here we attempt to throw some light on the utility of flow around the ring for digestive functions by monitoring both growth and energy conversion during long-term occlusion of either the RAo or LAo in alligators and caimans. The rationale for these experiments is that if the ring functions as suggested then, over time, blood will be able to get 'from here to there' regardless of its origin, so that the effects of any occlusion will be limited. If flow around the ring or

Theme edition of Australian Zoologist "*Ecology meets Physiology*", a Gordon Grigg festschrift, edited by Lyn Beard, Daniel Lunney, Hamish McCallum and Craig Franklin. Australian Zoologist Vol 35 (2) 2010.

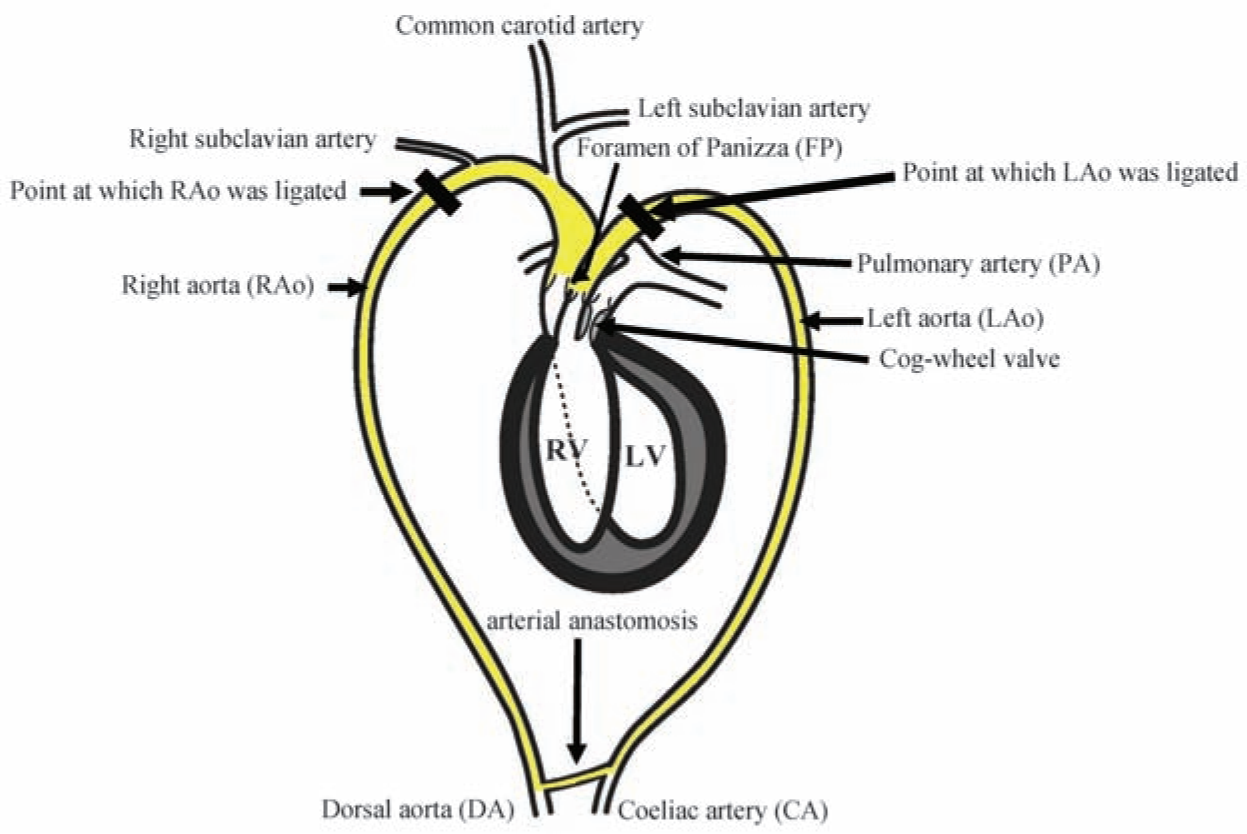

Figure 1. Diagram of the central cardiac anatomy of a juvenile alligator. The 'ring around the heart' is highlighted. The approximate position at which the LAo and RAo were ligated is indicated by the thick bars.

reverse flow through the FP occur as a last resort during prolonged underwater periods, we would not expect to find any drowned animals as only the integrity of the ring is breached so the FP can still function as a 'life saver'.

Materials and Methods

Alligators (*Alligator mississippiensis*) and caimans (*Caiman crocodilus*) were purchased from commercial suppliers and air freighted to Vancouver, British Columbia. The species were separated and housed in large rooms containing a pool with continuously flowing water. Water temperature was kept at 29.9 ± 0.8 °C (mean ± SD) and average room temperature was 26.0 ± 2.2 °C. A 12-hour light and 12-hour dark photoperiod was maintained throughout the studies. Animals were fed pieces of chicken, fish or whole mice and rats. Food was enriched with a multivitamin powder and Vitamin E.

All surgical procedures and experiments were approved by the University of British Columbia Committee on Animal Care. All Animals were fasted at least 48 hours before surgery. Surgical anesthesia was induced by inhalation of isoflurane in oxygen using a facemask. A surgical level of anesthetic was maintained by inhalation of 0.8% - 2% isoflurane in oxygen via an endotracheal tube. An incision was made in the ventral midline to expose the heart and to locate the major vessels. In six alligators, the LAo was tied off upstream and downstream (about 2 mm apart) and sectioned between the sutures (C; Fig 1). Sham surgeries were performed on five alligators (S). In three caimans, the LAo was sectioned between upstream and downstream ligatures (LAoC) while in three others the RAo was sectioned between ligatures sited downstream of the carotid and subclavian arteries (RAoC; Fig 1). The sternum and skin incisions were closed using nylon monofilament sutures and made watertight using cyanoacrylate glue. Hence there were two groups of alligators (C and S) and two groups of caimans (RAoC and LAoC).

After surgery, the animals were ventilated with 100% oxygen until they were awake and alert. The animals were put into a dry tank and allowed to recover for 48 hours. Baytril (antibiotic) was given intramuscularly twice, at three day intervals, to prevent any post-surgical infection of the incision. After two days the animals were returned to the water-filled holding tank. All animals were left to recover in the holding tank for at least five weeks before any measurements were made.

Mass (kg) of the two groups of caimans (RAoC and LAoC) and mass (kg) and length (m) of the alligator groups (S and C) was obtained every week just before feeding. To determine water intake, alligators were weighed twice, before and immediately after feeding. The difference between the amount of food eaten per group and the total amount of mass gained per group was taken as a measure of water intake with the food.

Individual growth rates were averaged for each group of alligators and caimans and recorded either as change in % or absolute mass per group per week. Food intake was measured either as % or absolute amount (g) of food eaten per total body mass per group per week. Conversion rates (*aka* digestive efficiencies) were calculated from growth rates divided by the mass of food eaten.

Head and body length measurements for caimans were made using digital calipers (see Verdade 2000 for measurement details). The measurements made were whole body length (BL), snout vent length (SVL), body width (BW), and an assortment of head size measurements such as dorsal cranial length (DCL), cranial width (CW), snout length (SL), snout width (SW), orbital length (OL) width (OW) and inter-orbital width (IOW) and mandible length (ML). These measurements were expressed as a percentage of snout-vent length and compared between LAoC and RAoC. Finally, we fitted our morphometric data to regressions presented by Verdade (2000) for captive *Caiman latirostris*.

Statistical analyses were performed using JMP (SAS Institute, Inc, Cary, NC, USA) and SigmaStat software (Jandel Corporation, Point Richmond, CA, USA). Percentage values were arcsine transformed before analysis. Unpaired t-tests were used to test for significant differences between groups. A significant difference was accepted at the level of $P < 0.05$. A generalized linear model was used for regression analysis and ANCOVA was used to compare slopes and intercepts. All values are presented as Mean ± Standard error of the mean (SEM).

Results

Effect of section of LAo and RAo on growth.

(1) Alligators:

At the start of these experiments the alligators were five months old. After recovery from surgery, growth was followed through 20 weeks of *ad lib* feeding and for 36 weeks while being fed 5% of body mass of the group (Fig 2a). The allometric growth equation for the whole 56 week period yielded the following relationship between body mass (M, kg) and length (L, m):

$$M = 3.69\ L^{3.03} \text{ for shams (S) (Fig 2a)}$$

$$M = 3.71\ L^{2.91} \text{ for LAo cut (C) (Fig 2a).}$$

When animals were fed *ad lib*, food intake varied by a factor of two from week to week. Average individual food intake per week was 3.0 ± 0.2 % for S and 2.9 ± 0.2 % for C and individual growth rates each week (S = 1.13 ± 0.15 % and C = 1.22 ± 0.18 %) were not significantly different (Fig 2b). Also, conversion rates in S (37 ± 4%) and C (42 ± 5%) were not significantly different.

When alligators were fed 5% of body mass per group per week, average individual food intakes were significantly different at 0.9 ± 0.03 % (S) and 0.8 ± 0.03 % (C) but average individual weekly mass gains were not significantly different (S = 0.30 ± 0.03 % and C = 0.25 ± 0.03 % per week; fig 2b). Conversion rates were 33 ± 2.9 % and 32 ± 2.3 % for S and C alligators, respectively and not significantly different.

Water intake (A) was somewhat proportional to the amount of food eaten but there was considerable variation in the relationship. On many occasions, both S and C consumed up to 0.3 kg of food (f) without any apparent water intake. In contrast, eating 0.5 to 0.8 kg of food could be accompanied by water intake of 0.1 to 0.25 l. The regression equation for C was $A = 0.28f - 89.4$ ($R^2 = 0.44$) and for S was $A = 0.35f - 75$ ($R^2 = 0.46$) where A = water intake and f = food intake. No differences between these regressions were significant.

(2) Caimans:

The caimans were about two years old at the start of these experiments. The LAoC had an average mass of 2.5 kg and the RAoC averaged 2.8 kg at the start of the growth experiment. Over the 20 week growth study, food intake was not significantly different between LAoC (88 ± 7 g $\text{caiman}^{-1}\text{week}^{-1}$) and RAoC (72 ± 9 g $\text{caiman}^{-1}\text{week}^{-1}$) (Fig 3). Both groups were offered 5% of group body mass to eat which was more than they actually consumed. Mass gain was also different but, again, not significantly. On average, each LAoC gained mass at 20 ± 6 g per week while in RAoC mass increased by only by 13 ± 7 g per week (Fig 3). Consequently, conversion efficiencies were lower in RAoC at 18% compared with LAoC at 22.5%.

The mass gain in four caimans surviving for a further 45 months was re-examined, having been fed *ad lib* over that period. Average body mass of the two RAoC survivors averaged 15 kg having gained in mass from the end of surgery at an average of 63 g $\text{caiman}^{-1}\text{week}^{-1}$ while the two LAoC survivors (average mass = 24kg) gained mass at nearly twice that rate (114 g $\text{caiman}^{-1}\text{week}^{-1}$).

In these four animals we also measured relationships between head and body length. SVL averaged 75 cm in LAoC and 61.5 cm in RAoC although the proportions of various head measures to SVL did not vary between LAoC and RAoC by more than one or two percentage points. For instance DCL averaged 26.6 % (LAoC) and 27.4 % (RAoC) of SVL; CW =19.7% and 20.2%; SL= 15.7% and 16.25%; SW= 13.8% and 16% and ML= 28.5 and 30.7% (all values given are LAoC and RAoC respectively).

Discussion

Section of the LAo, preventing bulk flow of venous blood to the systemic circulation is surprisingly without effect on either mass gain or conversion efficiencies in alligators. Section of the RAo in caimans had effects on both variables but, unfortunately, no differences were significant either because of extreme variability between animals or lack of adequate numbers. However, over the long term (45 months) caimans with the LAo cut gained mass at a rate nearly twice that of caimans with their RAo cut but there were not enough animals for any attempt at statistical verification because one animal from each group died. There were no ‘shams’ in the caiman experiments and section of the LAo was used as the “control”. In alligators this is without marked effects but we do not know if this is also the case in caimans.

Growth in crocodilians can be adequately described by the von Bertalanffy growth model (Moulton *et al* 1999) although segmented linear models have also proven useful (Webb *et al* 1983; Magnusson and Sanaiotti 1995). Many growth curves for crocodilians in captivity cover

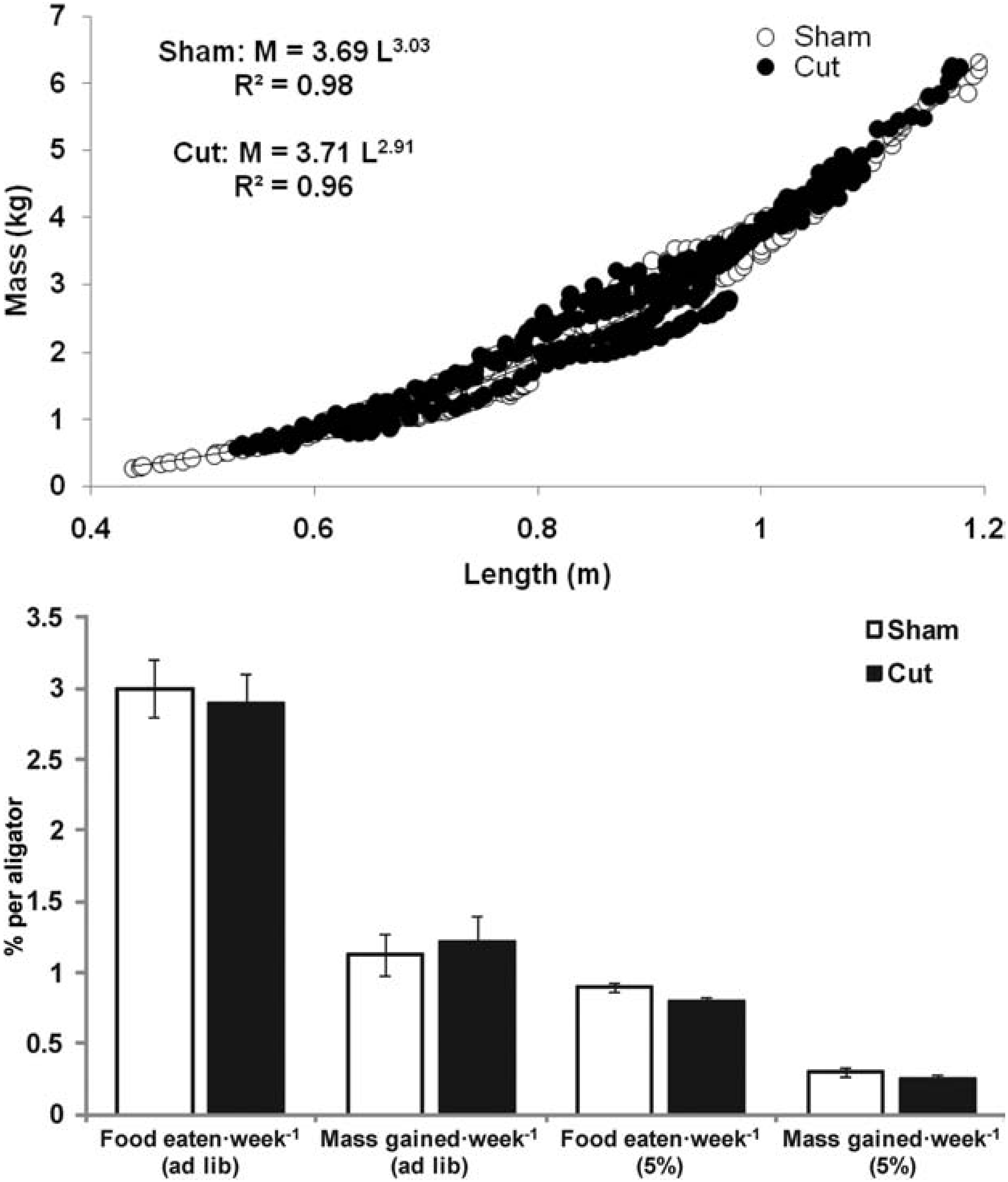

Figure 2 A and B. A - Length-mass relationship for juvenile alligators. Allometric equations for S and C are shown above the curves. B – Food intake per week and corresponding mass gain per week for S and C alligators when fed ad lib for 20 weeks and when offered 5% of total body mass in food for 36 weeks. Statistically significant differences between S and C are indicated by an asterisk.

only the first year of growth (Pina and Larriera 2002) although a number of species have been examined over several years from hatching (Herbert *et al* 2002). In captivity growth is affected by food type and quality as well as issues with temperature fluctuations, lighting and rearing density (Poletta *et al* 2008). Our animals were all the same age, feeding and lighting was controlled and density was much lower than that seen in commercial culture.

The growth curve of both C and S alligators was a power function with mass related cubically to length which is to be expected in animals which retain the same body shape from hatching to maturity. There were no significant differences between C and S alligators in growth. Even better, growth was effectively linear in alligators when food intake was restricted which is ideal for a physiologist conducting a growth experiment!

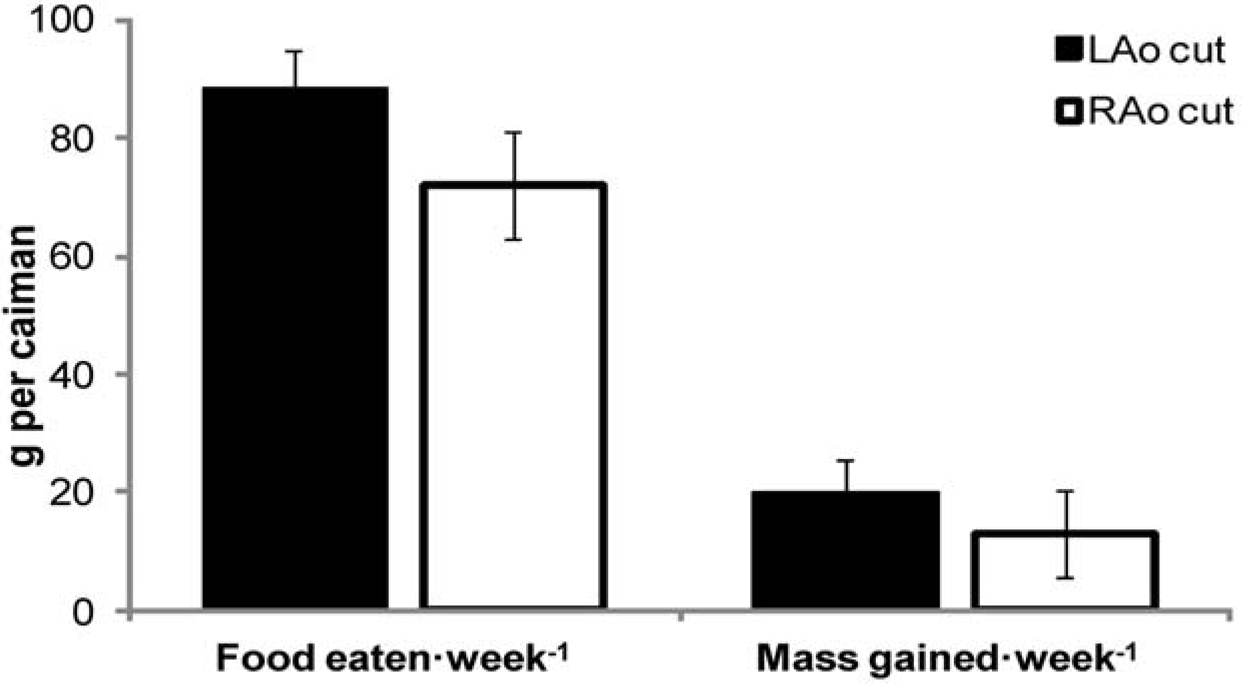

Figure 3. Average food intake per week and corresponding mass gained per week for RAoC and LAoC caimans offered 5% of body mass in food for 20 weeks.

In caimans, by closing off the RAo, have we interfered with digestive, absorptive and mobilization processes or is it simply that we have cut off the supply of oxygen to a major growing region of the body? That two animals survived after RAoC surgery for four years does not indicate a generalized systemic effect. Furthermore, proportionate relationships between various cranial lengths and SVL for caimans showed no differences between LAoC and RAoC. Verdade (2000) presented a much more detailed analysis of these proportionate relations for *Caiman latirostris* and our measures fitted his regressions 'bang-on' for SVL and BL, SVL and SW and SVL and ML, the latter two regressions being cranial measures. Since the cranial end presumably received an adequate supply of oxygenated blood why did it not grow faster than the hind end? Growth obviously remains proportionate regardless. Consequently, we confidently conclude that, at the very least, we have developed a technique to 'bonsai' crocodilians.

The patterns of blood flow established after section of LAo and RAo are unknown. In untreated or sham operated animals, flow in the ring is usually driven by output from right and left ventricles in every cardiac contraction but, during acute experiments, this is a much less frequent occurrence and flow originates largely from LV output. However, in chronic experiments it has been shown that operation of the pulmonary by-pass shunt is normal rather than exceptional (Jones 1996; Axelsson *et al* 2006). RV blood is shunted into the LAo in the latter part of systole after occlusion of the pulmonary outflow tract by closure of the cog-wheel valve and, only then, if pressure in the LAo exceeds that in the systemic circulation (Fig.1; Jones and Shelton 1993). Grigg (1991) described the "nubbins of tissue" around the pulmonary outflow tract as the "nubbins" of the issue of shunting, which is indeed the case. The "nubbins of tissue" is the cog-wheel valve that completely occludes the PA in mid-systole and if there is enough blood left in the RV to allow generation of a pressure above that in the RAo then it will be shunted into the LAo (Syme *et al* 2002). Inactivation of cog-wheel function prevents the shunt except during extreme pulmonary vasoconstriction, when pulmonary pressure is higher than systemic, and PA flow and the shunt occur together throughout systole (Axelsson *et al* 1989; Shelton and Jones 1991; Syme *et al* 2002) although flow in the RAo falls due to a reduction of pulmonary venous return (Shelton and Jones 1991).

Despite the attention lavished on the pulmonary by-pass shunt by way of the LAo, showing any kind of selective advantage for retention of the shunt in the face of a completely divided heart has proven elusive. Farmer and Hicks (2002) have detailed at least eight hypotheses for the role of the RV→LAo shunt in reptiles although only a couple of these have any strong experimental support. Usually, the RV→LAo shunt results in venous blood by-passing the lungs, delivering blood rich in CO_2, H^+ and HCO_3^- to the systemic circulation. Really, it seems that use of venous blood to promote digestion is a "no brainer" in an animal that gorges infrequently and a clear indication that venous blood promotes digestion in terms of gastric acid secretion and rate of bone dissolution has been given recently by Farmer *et al* (2008). Nevertheless, in our alligators prevention of bulk flow of venous blood by LAo section had no overt effect on either growth or digestive efficiency. Anecdotal observations have remarked on the enormity of the crocodilian appetite and the capacity of the crocodilian gut but, in a controlled experiment, the animals are provided reasonable portions on a regular basis, which are unlikely to overstress the digestive system.

There is universal agreement that the vertebrate gut is an aerobic organ and, if shunting is promoted post-prandially (Farmer *et al* 2008), is sufficient oxygen available from venous blood? Farmer *et al* (2008) suggested that flow from RAo to LAo during systole is a requisite to provide an oxygen pulse for metabolic functions in the digestive system. The extreme growth limitation after RAo occlusion in caimans confirms that a supply of oxygenated blood from the RAo is indeed necessary for growth but, as is confirmed by LAo occlusion, it is not satisfied by RAo→LAo flow through the FP during systole. So how does oxygen get to the gut under normal circumstances? If the AN is patent then in non-shunting as well as many shunting conditions there is a pulse of oxygenated blood delivered to the LAo and gut circulation at the start of systole, through the anastomosis (Shelton and Jones 1991; Jones and Shelton 1993). If the AN is closed, and the shunt is not engaged, then a pulse of oxygenated blood may flow through the FP when the aortic valves open at end systole because pressure in the LAo will be low and the two circulatory volumes being connected through the FP are substantial (Jones1996). Certainly, new insights on the formation of the FP in the late embryo have served to underline its importance in crocodilians (Seymour *et al* 2004). Nevertheless, it seems unlikely that adequate oxygen would be supplied to an active gut from the RAo either through the AN or FP so perhaps the alligator gut is less aerobic than we thought.

How about reverse flow through the FP as a life saver in situations where all the blood is shunted away from the lungs and there is nothing for the RV to pump? By their design, these experiments can shed no light on this problem because the foramen was not tied off so LAo→RAo flow is always an option. No animals were drowned in these experiments although all were given ready access to a pool filled with water. Perhaps our animals never made dives of sufficient length but if one did (perhaps a larger one sat on it) the unfortunate animal survived! The RAo valves completely cover the FP during systole, although the LAo valves do not, so when working properly, blood can flow only from the LAo to the RAo during diastole after the RAo valves close (Axelsson *et al* 1996). The foramen is surrounded by smooth muscle and richly innervated, suggesting that the diameter may be of variable size depending on physiological state (Grigg and Johansen 1987; Grigg 1989; Axelsson and Franklin 2001). It is entirely possible that flow could occur in systole if the foramen was extremely dilated especially in the presence of a reduced right aortic valve. In fact, Malvin *et al* (1995) used hydrogen electrodes to look at different shunting patterns and LAo→RAo foramen flow was seen in one animal both under control conditions and during vagal stimulation. Much has been made of the observation that the FP is not always occluded in systole (Hicks 2002) despite only being recorded in a single animal (Malvin *et al* 1995). On the other hand, flow through the FP in systole has never been conclusively shown in pressure and flow studies (Jones and Shelton 1993). Occlusion of the PA, preventing any flow to the lungs, induces a substantial R→L shunt while flow in the RAo is greatly reduced. During PA occlusion, a large systolic pressure difference develops between LAo and RAo which could not be maintained with any substantial connexion across the FP. At end systole, the foramen opens and LAo pressure falls rapidly until reaching that in the RAo when both pressures decline together in diastole. In this case, even a very small amount of flow in the RAo in systole served to open the right aortic valve and close off the foramen. Certainly in the present experiments venous blood had access to the brain through the FP in both LAoC and RAoC and if an extreme situation arose then cerebral blood flow could have been satisfied in all cases, as was indeed the case.

The "ring around the heart" concept goes some way to clarify how blood leaving the crocodilian heart can move from RAo→LAo and vice-versa. Nevertheless, the over arching theme of a crocodilian central circulatory system which is extremely complex remains. This was clear from much previous work even starting with the classic paper of Greenfield and Morrow (1961) and these present experiments have given us little direction for unraveling these complexities. Certainly, showing a selective advantage for retention of the R→L and L→R shunts by the approaches we used produced only equivocal results, although growth seems better served by an adequate supply of oxygenated blood to the lower body. Grigg (1991), at his enigmatic best, set up a conundrum requiring an interventionist rather than serendipitous approach to show that LAo→RAo flow could be a 'life-saver' in long anaerobic dives. Nevertheless, advances in remote monitoring of behavioural and physiological variables in nature suggest that an attempt to test this idea may come sooner rather than later. At the very least, one thing we have accomplished by this study is further bolstering of the crocodilian reputation for *schadenfreude* with we, as well as the crocodilians, "teary-eyed"!

Dedication

This paper is dedicated to Gordon Grigg on the occasion of his retirement. Gordon has amused, engaged and occasionally even enraged with his unique insights into comparative and ecological physiology. His ability to look at problems that we have all mulled over for years and to come up with ingenious solutions, followed by novel experiments to prove his hypotheses, has been his own, very special hallmark. However, there is more to life than science! What makes Gordon really special is that he is a wonderful person to be around; funny, kind, helpful and compassionate, traits worthy of continual celebration. Health and happiness, Gordon!

Acknowledgements

We thank Arthur Vanderhorst and the staff at the UBC Animal Care Centre for their help in caring for the animals. Funding for this study was provided by an NSERC Discovery Grant awarded to David R. Jones.

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APPENDIX I

Caimans watching me cautiously, curiously.

Photo, M. Gardner.

APPENDIX I

First feeding after arriving in the lab.

Photo, M. Gardner.

Hungry gator. Feeding time was always a fun and challenging time.

Photo, M. Gardner.

Lucy is an amazing alligator that I was able to place in a rescue center/ zoo. The place is called Croctalk and is in Kelowna, Canada. She is truly a beautiful animal and I became rather attached to her and do miss her.

Photo, M. Gardner.

Lucy "smiling" at me, waiting for food.

Photo, M. Gardner.

Feeding frenzy.

Photo, M. Gardner.